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Ventricular myocarditis coincides with atrial myocarditis in humans

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ABSTRACT

Aims: The inflammatory response in myocarditis critically determines remodelling of the heart as well as the outcome. A common complication in myocarditis is atrial fibrillation (AF), which is associated with inflammation of the atria. However, research regarding the inflammatory response after myocarditis was focused almost exclusively on the ventricles of the heart. Therefore, in this study, we analyzed the inflammatory response in the atria in patients with lymphocytic myocarditis or catecholamine-induced myocarditis both without AF.

Methods: From post-mortem obtained hearts of patients with lymphocytic myocarditis (n = 6), patients with catecholamine-induced myocarditis (n = 5), and controls (n = 5), tissue slides of the left and right ventricles and left and right atria were stained with antibodies identifying neutrophilic granulocytes, lymphocytes, and macrophages. These cells were subsequently quantified in atrial and ventricular myocardium and atrial adipose tissue.

Results: In patients with lymphocytic myocarditis, neutrophilic granulocytes in the right atrial myocardium and lymphocytes in the left atrial adipose tissue were significantly increased. In patients with catecholamine-induced myocarditis, a significant increase was observed for neutrophilic granulocytes in the myocardium of both atria and the right atrial adipose tissue, for lymphocytes in the left atrial adipose tissue and the right atrial myocardium, and for macrophages in the myocardium of both atria. The numbers of infiltrating inflammatory cells in the atrial myocardium correlated positively with those in the ventricles, especially in patients with catecholamine-induced myocarditis.

Conclusion(s): To a varying extent, atrial myocarditis occurs concurrently with ventricular myocarditis in patients diagnosed with myocarditis of different aetiology. This may explain their predisposition to atrial complications such as AF.

INTRODUCTION

Myocarditis is defined by inflammation of the heart and is a major underlying cause of heart failure and sudden death. Myocarditis is characterized by a heterogeneous aetiology and pathophysiology. The most common and well-described form of myocarditis is lymphocytic myocarditis, in majority related to viral infection¹⁻⁵. A non-infectious form of myocarditis, which over the past 20 years has become a clinical topic of increasing interest, is catecholamine-induced myocarditis, which is found in 62% of patients diagnosed with stress-induced cardiomyopathy^{6,7}. Although the mechanisms underlying catecholamine-induced myocarditis are still not well understood, an increased infiltration of different inflammatory cells was described in human ventricular heart tissue. Indeed, Yoshida *et al.* observed mononuclear cell infiltration in endomyocardial biopsy (EMB) samples taken from the left ventricle of three patients with stress-induced cardiomyopathy⁸, while Nef *et al.* described increased macrophages and lymphocytes in the right ventricle of patients with stress-induced cardiomyopathy⁹. However, both the diagnosis as well as research into myocarditis are focussed almost entirely on the ventricles of the heart, whereas very little is known about how myocarditis affects the atria.

A common cardiac complication in patients with myocarditis is atrial fibrillation (AF). There is strong evidence linking both systemic and local inflammation to the initiation and perpetuation of AF in general^{10,11}. For instance, increased infiltration of macrophages¹² and lymphocytes¹³ has been described in the left and right atria of patients with symptomatic atrial fibrillation (AF), compared to control patients. In addition, AF shares strong epidemiological associations with other cardiovascular diseases wherein cardiac inflammation plays an important pathogenic role, such as hypertension, valvular disease, myocardial infarction, and myocarditis¹⁴. These studies suggest that (cardiac) inflammation predisposes to the onset of AF. However, it is not known whether ventricular myocarditis coincides with infiltration of inflammatory cells in the atria (referred to as atrial myocarditis) in patients with myocarditis. The presence of atrial myocarditis in patients with myocarditis, may predispose these patients to the development of AF.

In this study, we quantitatively analyzed neutrophilic granulocytes, lymphocytes and macrophages in the atria and ventricles of patients with lymphocytic and catecholamine-induced myocarditis.

METHODS

Patients

This study was approved by and performed according to the guidelines of the ethics committee of the VU Medical Center (VUmc), Amsterdam, the Netherlands, in concordance with the guidelines established by the World Medical Association (Declaration of Helsinki). Human heart tissue was obtained at autopsy at the Pathology department of the VUmc and used in this study retrospectively. In the VUmc, as part of the patient contract, or in case relatives have given explicit prior written consent, tissue taken at autopsy can be used for research after completion of the diagnostic process. From each patient tissue from the left ventricular anterior wall, the right ventricular lateral wall, and the left and right atrial appendages (auricles) of the heart was obtained for analysis.

Details of the included patients are listed in *table 1*. The patient groups did not differ, between each other, in gender or age. Patients were diagnosed post-mortem with lymphocytic myocarditis based on presence of aggregates of lymphocytes adherent to cardiomyocytes, in combination with myocytolysis (objectified as complement factor 3d-positivity of cardiomyocytes) in the ventricular myocardium. Instead, patients were diagnosed with catecholamine-induced myocarditis when myocytolysis coincided with a diffuse infiltration of neutrophilic granulocytes, macrophages, and lymphocytes in the ventricular myocardium rather than aggregates of lymphocytes. In addition, control patients were included without pathological evidence of heart disease. A lactate dehydrogenase (LDH) staining was performed on a ventricular cross sectional heart slice from each patient, to detect putative infarctions. As this study aims to investigate atrial myocarditis as a possible predisposing factor for AF, only patients were selected who did not have symptomatic AF. Patients with infarctions were excluded from this study.

Table 1. Patient characteristics

Patient	age	sex	Primary cause of death	Secondary Pathologies	Blood leukocyte count	PMI	PC
<i>Control</i>							
1	67	M	Respiratory insufficiency	-	13,1•10 ⁹ /l	24h	C
2	66	F	Respiratory insufficiency	Adenocarcinoma (m)	7,0•10 ⁹ /l	18h	A B
3	71	M	Euthanasia	Adenocarcinoma (m) Pneumonia DM type 2	20,9•10 ⁹ /l	24h	
4	35	F	Hypovolemic circulatory shock (ruptured aorta)	Marfan syndrome	Unknown	40h	
5	57	M	Hypovolemic circulatory shock (ruptured aorta)	-	9.6•10 ⁹ /l	24h	
<i>Lymphocytic Myocarditis</i>							
1	59	F	Lymphocytic myocarditis	Leiomyosarcoma (m) Pneumonia	18.1•10 ⁹ /l	18h	
2	28	F	Lymphocytic myocarditis		Unknown	21h	
3	73	F	Hypovolemic circulatory shock (external wound)	Lymphocytic myocarditis COPD	Unknown	<12h	
4	32	M	Lymphocytic myocarditis	DM type 1	Unknown	24h	
5	46	M	Lymphocytic myocarditis	Morbus Crohn	9.5•10 ⁹ /l	24h	
6	40	M	Lymphocytic myocarditis	Sarcoidosis (lungs)	14.0•10 ⁹ /l	24h	
<i>Catecholamine-induced Myocarditis</i>							
1	80	M	COPD	Catecholamine myocarditis	8.8•10 ⁹ /l	36h	
2	60	F	Carcinoma (m)	Catecholamine myocarditis Spinal fracture	11.6•10 ⁹ /l	20h	
3	80	F	Hypovolemic circulatory shock (ruptured aorta)	Catecholamine myocarditis	14.9•10 ⁹ /l	24h	
4	56	M	Gastritis	Catecholamine myocarditis Adenocarcinoma DM type 2	13.1•10 ⁹ /l	60h	
5	52	M	Brainstem ischemia	Catecholamine myocarditis Bronchitis	11.1•10 ⁹ /l	20h	

Abbreviations: (m), tumour with metastases; COPD, Chronic Obstructive Pulmonary Disease; DM, Diabetes mellitus; F, female; M, male; PC, Possible confounders; PMI, Post-mortem interval.

Possible confounders: A. Patient was using an anti-inflammatory drug prior to death (dexamethasone). B. Patient had tumor metastases in the right adrenal gland, which may have affected catecholamine production. C. Patient was using an anti-inflammatory drug prior to death (prednisone).

Immunohistochemistry

For immunohistochemical analysis, formalin-fixed paraffin-embedded tissue was cut into 4 μm sections, deparaffinised, dehydrated, and incubated in methanol/ H_2O_2 (0,3%) for 30 minutes to block endogenous peroxidases. Antigen retrieval was performed by heat inactivation in sodium-citrate buffer (10 mM, pH 6.0) for slides to be stained for myeloperoxidase (MPO; neutrophilic granulocytes) and CD68 (macrophages). Slides stained for CD45 (lymphocytes) did not require antigen retrieval. The slides were incubated with either rabbit anti-human MPO (1:700, Dako Cytomation, Eindhoven, The Netherlands), mouse anti-human CD68 (1:400, Dako Cytomation) or mouse anti-human CD45 (1:50, Dako Cytomation) for 1 hour at room temperature. The sections were then washed with phosphate-buffered saline and incubated with RealTM EnVisionTM HRP α -mouse/rabbit (undiluted, Dako Cytomation) for 30 minutes at room temperature. The staining was visualized using 3,3'-diaminobenzidine (0.1 mg/ml, 0.02% H_2O_2). Next, the sections were counterstained with hematoxylin, dehydrated, and covered. With each staining a phosphate buffered saline control and an isotype control were included for each antibody. All these controls yielded negative results (data not shown).

Quantitative immunohistochemical analysis

The extravascular MPO-positive, CD68-positive and CD45-positive cells were quantified in the myocardium of the left and right ventricle and the left and right atria of the heart. Recently, we observed in left atrial appendage tissue of AF patients that inflammatory cells also infiltrated the adipose tissue (unpublished results). Therefore, in this study, we analysed the atrial adipose tissue separately. In the atria we identified areas of cardiomyocytes as myocardium and areas of adipose tissue as adipose tissue, regardless of whether this adipose tissue was part of the epicardium or embedded in the myocardium. In one lymphocytic myocarditis patient the adipose tissue was not analysed, because the atrial tissue slides contained an insufficient amount of adipose tissue ($<0.3 \text{ mm}^2$). The total surface area of each sample was measured using QPRODIT and the numbers of cells per mm^2 were calculated as the total score for each specimen.

Collagen staining and quantification

Atrial collagen was visualized histochemically using a Picrosirius Red staining. Formalin-fixed paraffin-embedded tissue was cut into 4 μm sections, deparaffinised, and rehydrated. The sections were incubated in a 0.2% Sirius Red solution (solution made in 1.2% picric acid; VWR International, Radnor, PA) for 60 min at room temperature. Subsequently, the staining was differentiated in 0.01N HCl for 2 minutes at room temperature. Following this, the sections were washed in tap water, dehydrated, and covered. For quantitative analysis, six photos were taken randomly throughout the myocardium of each atrial tissue section, using a Leica DM/RB microscope equipped with a polarization filter. ImageJ software version

1.63 (NIH, Bethesda, MD: <http://imagej.nih.gov/ij/>) was used to calculate the percentage of collagen on each photo and the mean percentage of all six photos was taken as the data value of an individual patient. The quantitative analysis was performed blinded.

Statistical analysis

Statistical analysis was performed using Prism 6.0 (GraphPad Software, La Jolla, CA). Differences in infiltrating inflammatory cell numbers per mm² and collagen deposition were calculated with the Mann-Whitney U-test, while correlations were determined using Spearman's rank correlation coefficient. A correlation coefficient ranging from $r \pm 0.10$ to 0.29 indicates absence of correlation, $r \pm 0.30$ to 0.49 indicates a medium correlation, and $r \pm 0.50$ to 1.0 indicates a strong correlation. Correlations were calculated separately for each myocarditis group. In addition, for the correlations we also calculated the coefficient of determination (cod). P-values ≤ 0.05 were considered significant for all analyses.

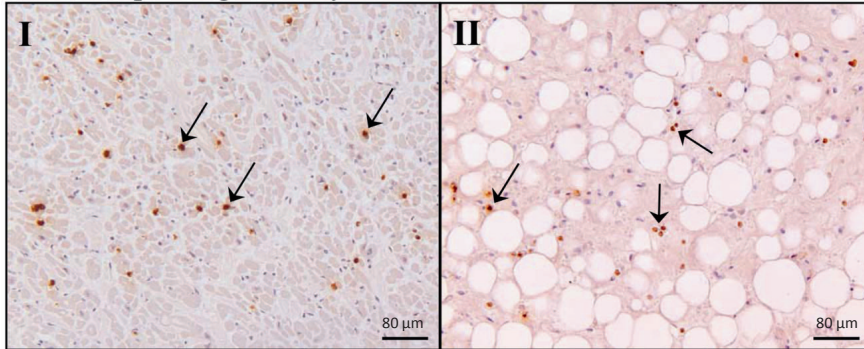
RESULTS

Infiltrating inflammatory cells in the atria of patients with myocarditis

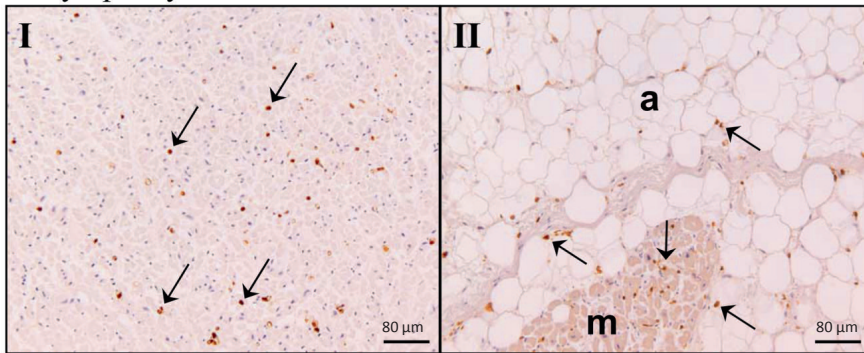
In general, a diffuse pattern of infiltrating neutrophilic granulocytes (figure 1A), lymphocytes (figure 1B), and macrophages (figure 1C) was observed in the atria of patients with myocarditis. All these inflammatory cells, were found to infiltrate both the atrial myocardium and the atrial adipose tissue.

In the atria of patients with lymphocytic myocarditis, focal aggregates of lymphocytes were present, similar to the ventricles. Besides that, there were no obvious differences in the pattern or the location of these infiltrating cells, between patients with lymphocytic myocarditis and patients with catecholamine-induced myocarditis.

A Neutrophilic granulocytes



B Lymphocytes



C Macrophages

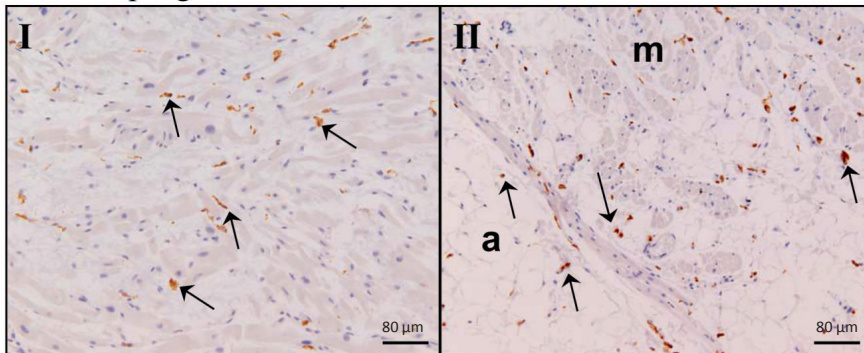


Figure 1. Infiltrated inflammatory cells in the atrial myocardium and atrial adipose tissue

Representative images of: (A) diffuse infiltration of MPO-positive neutrophilic granulocytes (arrows) in the atrial myocardium (left image (I)) and atrial adipose tissue (right image (II)) in the left atrium of a patient with catecholamine-induced myocarditis; (B) diffuse infiltration of CD45-positive lymphocytes (arrows) in the left (I) and right atrial myocardium (II, m) and the right atrial adipose tissue (II, a) of a patient with lymphocytic myocarditis; and (C) diffuse infiltration of CD68-positive macrophages (arrows) in the right (I) and left atrial myocardium (II, m) and the left atrial adipose tissue (II, a) of a patient with catecholamine-induced myocarditis (I) and a patient with lymphocytic myocarditis (II).

Quantification of infiltrating inflammatory cells in patients with lymphocytic myocarditis

Neutrophilic granulocytes

In the patients with lymphocytic myocarditis, an increase in neutrophilic granulocytes was found in the myocardium of the left and right ventricle and to a lesser extent also in the left and right atrium, compared to controls (figure 2A). However, none of these increases were found to be statistically significant. In the adipose tissue of the left and right atrium, no significant differences in the number of neutrophilic granulocytes were found, compared to controls (figure 2A). Also, no significant differences were found in the number of neutrophilic granulocytes between the ventricles and the atria (figure 2A). Correlation analysis revealed a strong correlation between the number of neutrophilic granulocytes in the left atrium and the left ventricle and a medium correlation between the adipose tissue and myocardium of the left atrium. No correlations were found on the right side of the heart (supplementary table 1).

Lymphocytes

Similar to neutrophilic granulocytes, patients with lymphocytic myocarditis showed an increase of lymphocytes in the myocardium of the left and right ventricle and the left and right atrium, compared to controls, which was significant for the right ventricle ($p = 0.0043$; figure 2B). In the adipose tissue of the left and right atrium, the number of lymphocytes was also increased, compared to controls, which was significant for the left atrium ($p = 0.0043$; figure 2B). Although no significant differences were found in the number of lymphocytes between the ventricles and the atria (figure 2B), a strong correlation was found between the number of lymphocytes in the right ventricle and right atrium (supplementary table 1).

Macrophages

The most abundant increase of inflammatory cells in the hearts of patients with lymphocytic myocarditis, was found for the number of macrophages. In the myocardium of the left and right ventricle and the left and right atria, a non-significant increase in the number of macrophages was found, compared to controls (figure 2C). Similarly, in the adipose tissue of the left atrium, a non-significant increase in the number of macrophages was also found, compared to controls (figure 2C). However, in the adipose tissue of the right atrium, no increase in the number of macrophages was found (figure 2C). In addition, no significant differences were found in the number of macrophages between the ventricles and the atria (figure 2C). Albeit, a strong correlation was found between the number of macrophages in the left ventricle and the left atrium, the right ventricle and the right atrium, and between the number of macrophages in the adipose tissue and the myocardium of both atria (supplementary table 1).

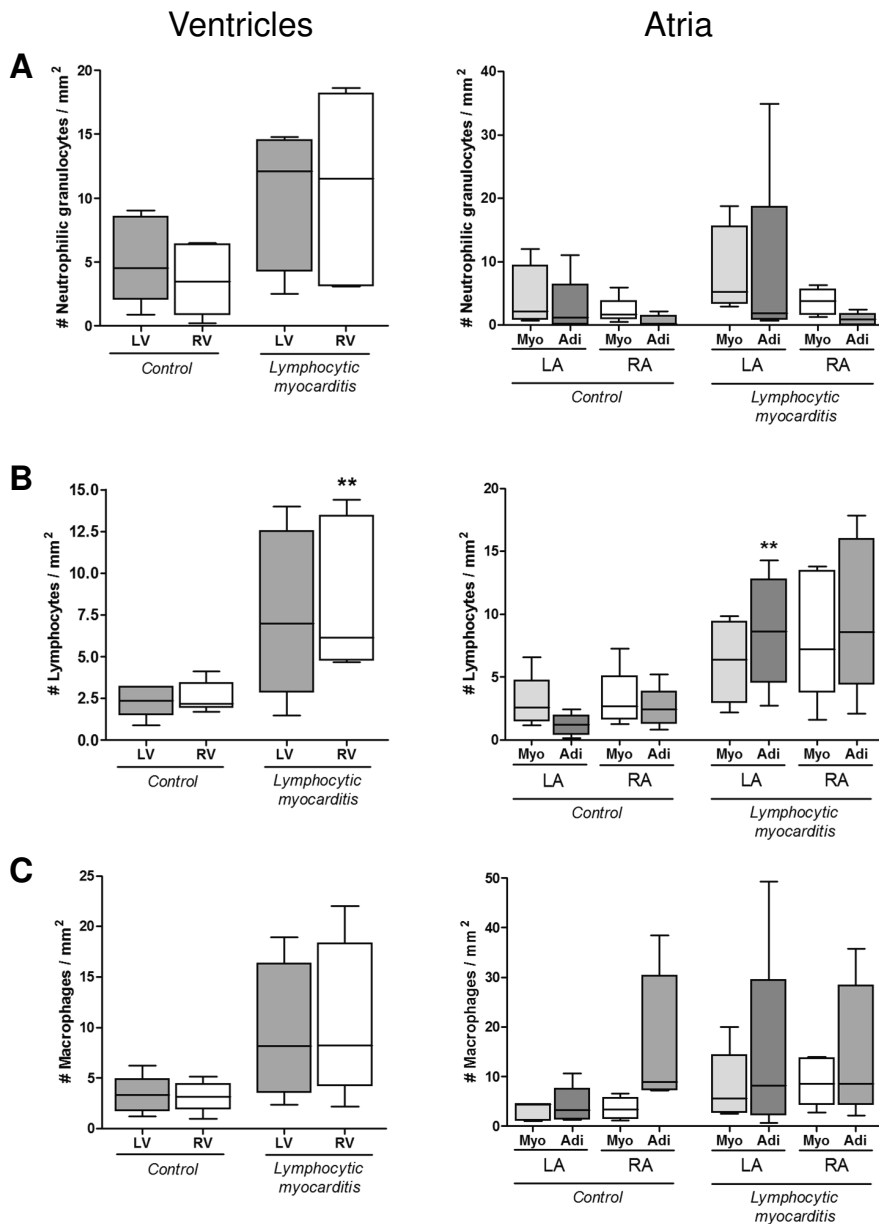


Figure 2. Infiltrated inflammatory cells in the hearts of lymphocytic myocarditis patients.

A-C: The number of neutrophilic granulocytes (**A**), lymphocytes (**B**) and macrophages (**C**) per mm², quantified in tissue obtained from the left and right ventricles (LV and RV; left graph) and the left and right atria (LA and RA; right graph) of control patients (n = 5) and patients diagnosed with lymphocytic myocarditis (n = 6). In the atria, the infiltrated inflammatory cells in the myocardium (Myo) and the adipose tissue (Adi) were quantified separately. Data are box-plots with whiskers representing minimum and maximum values, the boxes the lower and upper quartiles, and the black lines the medians. ** p < 0.01 compared to the corresponding cardiac compartment in controls.

Quantification of infiltrating inflammatory cells in patients with catecholamine-induced myocarditis

Neutrophilic granulocytes

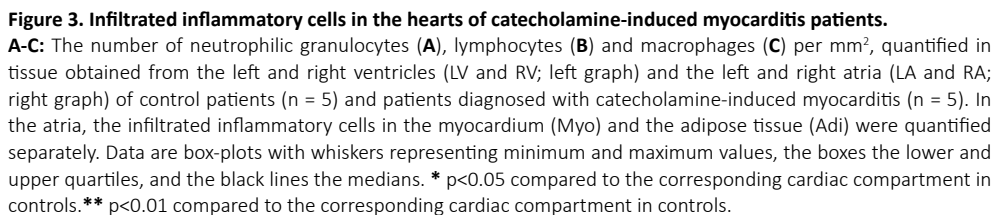
In patients with catecholamine-induced myocarditis, an increase in neutrophilic granulocytes in the myocardium of the left and right ventricle and the left and right atrium was found, compared to controls, which was significant for the right atrium ($p = 0.016$; figure 3A). In the adipose tissue of the right atrium, the number of neutrophilic granulocytes was also significantly increased, compared to controls ($p = 0.032$; figure 3A). Although no significant differences were found in the number of neutrophilic granulocytes between the ventricles and the atria (figure 3A), a strong correlation was found between the number of neutrophilic granulocytes in the right ventricle and right atrium and between the number of neutrophilic granulocytes in the adipose tissue and the myocardium of the left atrium (supplementary table 2). A medium correlation was found between the number of neutrophilic granulocytes in the left ventricle and left atrium (supplementary table 2).

Lymphocytes

In patients with catecholamine-induced myocarditis, an increase in the number of lymphocytes was found in the myocardium of the left and right ventricle and the left and right atrium, compared to controls, which was significant for the left ventricle ($p = 0.0079$; figure 3B). In the adipose tissue of the left and right atrium, increased numbers of lymphocytes were also found, compared to controls, which was significant for the left atrium ($p = 0.016$; figure 3B). The number of lymphocytes were also significantly higher in the left ventricle, compared to the left atrium ($p = 0.032$), albeit, no correlations were found between the number of lymphocytes in the ventricles and the atria and between the adipose tissue and the myocardium of the atria (supplementary table 2).

Macrophages

In patients with catecholamine-induced myocarditis, significantly increased numbers of macrophages in the myocardium of the left and right ventricle (both $p = 0.0079$) and the left and right atrium ($p = 0.0079$ and $p = 0.0159$, respectively) were found, compared to controls (figure 3C). In the adipose tissue of the left atrium, a large, but non-significant increase in the number of macrophages was also found (figure 3C). Moreover, no significant differences were found in the number of macrophages between the ventricles and the atria (figure 3C). However, a strong correlation was found between the number of macrophages in the left ventricle and the left atrium, the right ventricle and the right atrium, and between the number of macrophages in the adipose tissue and the myocardium of both atria (supplementary table 2).



Notably, no significant differences were found in the numbers of neutrophilic granulocytes, lymphocytes and macrophages in the atria between patients with lymphocytic myocarditis and patients with catecholamine-induced myocarditis.

Atrial fibrosis

It is known that atrial inflammation can stimulate the formation of atrial fibrosis, which by itself increases the risk of AF development¹⁵. Therefore, we determined whether the increased inflammatory cell numbers we observed in the atria of patients with myocarditis, included in the present study, were also accompanied by an increased amount of collagen in the atrial myocardium. In the right atrium of patients with lymphocytic myocarditis, we found a decreased amount of collagen compared to control patients and patients with catecholamine-induced myocarditis (figure 4A). However, when comparing the percentages of collagen in the myocardium of patients with lymphocytic and catecholamine-induced myocarditis with control patients, no significant differences were found in both atria (figure 4A). Interestingly, for all three patient groups, we found an increased amount of collagen in the right atria, compared to the left atria of the same patient group (figure 4B), which was significant for control patients ($p = 0.032$) and patients with lymphocytic myocarditis ($p = 0.026$, figure 4A).

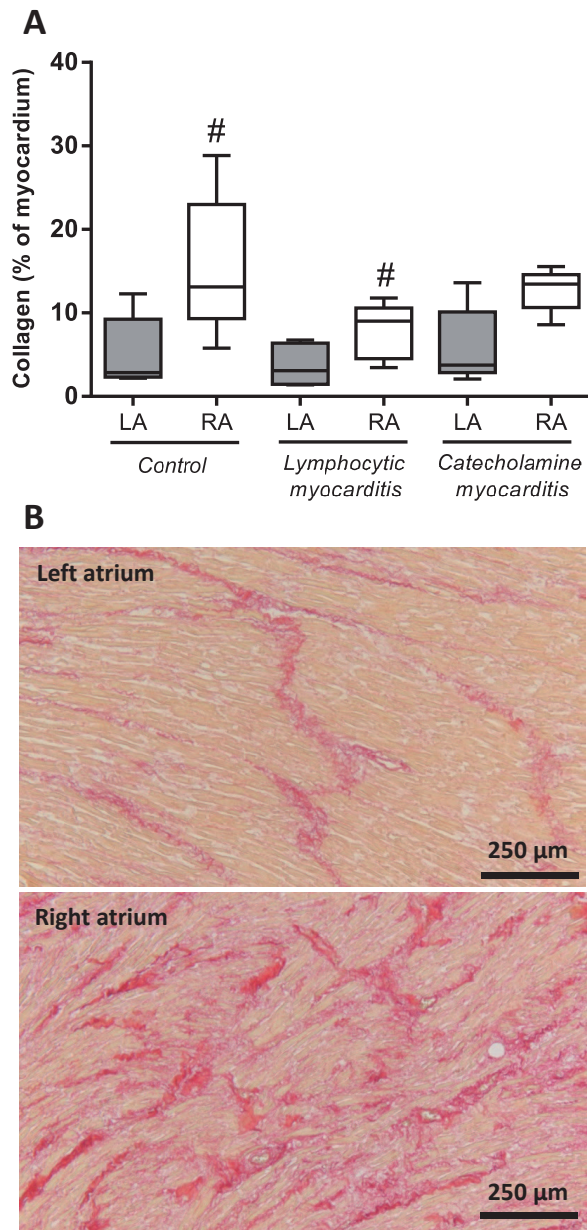


Figure 4. Collagen quantity in the atrial myocardium

A: Quantity of collagen as the percentage of the atrial myocardium in the left atrium (LA) and right atrium (RA) of control patients ($n = 5$), patients with lymphocytic myocarditis ($n = 6$), and patients with catecholamine-induced myocarditis ($n = 5$). Data are box-plots with whiskers representing minimum and maximum values, the boxes the lower and upper quartiles, and the black lines the medians. [#] $p < 0.05$ compared to the left atrium.

B: Collagen staining (red) of the left and right atrium of a patient with catecholamine-induced myocarditis.

DISCUSSION

To the best of our knowledge, this study, is the first to describe in detail infiltration of inflammatory cells in the atria of patients diagnosed with myocarditis. In patients with lymphocytic myocarditis, we only found a significant increase in the number of lymphocytes in the left atrial adipose tissue. In patients with catecholamine-induced myocarditis, the atrial inflammation appeared more prominent. A significant increase was found for the number of neutrophilic granulocytes in the myocardium and adipose tissue of the right atrium, for the number of lymphocytes in the left atrial adipose tissue, and for the number of macrophages in the myocardium of both atria. The numbers of infiltrating inflammatory cells in the atrial myocardium correlated positively with those in the ventricles, especially in patients with catecholamine-induced myocarditis.

Myocarditis is commonly diagnosed via (immuno)histological analysis of endomyocardial biopsies taken from either the left or right ventricle of the heart. Inflammation of the atria, however, was never before explored in patients with myocarditis. Atrial myocarditis was observed before in atrial tissue obtained post-mortem from young patients after sudden death¹⁶ and in atrial tissue obtained from living patients with idiopathic enlargement of bilateral atria¹⁷, with transient sinoatrial disease¹⁸ and with atrial giant cell myocarditis¹⁹. In all these cases, however, atrial myocarditis occurred in the absence of ventricular myocarditis. We now show that atrial myocarditis also occurs in patients diagnosed with ventricular myocarditis.

Atrial myocarditis coincided with ventricular myocarditis of different aetiology, i.e. lymphocytic myocarditis, which in majority is caused by viral infection and/or resulting from autoimmunity, and catecholamine-induced myocarditis. In our study, atrial inflammation appeared most pronounced in patients with catecholamine-induced myocarditis, although the extent of the inflammatory infiltrate did not differ significantly between both myocarditis patient groups. Because local catecholamine overload is considered an important cause of ventricular catecholamine-induced myocarditis²⁰, such catecholamine overload may underlie atrial myocarditis as well, even more since adrenergic receptor-protein coupling in human atrial myocytes appears to be similar to ventricular cardiomyocytes²¹. In addition, in catecholamine-induced myocarditis an elevated blood pressure is expected, as high levels of catecholamines can result in hypertension²². In turn, this could result in pressure overload and stretching of cardiomyocytes in the heart, including the atria. Stretching of atrial cardiomyocytes has been shown to promote inflammation²³. Interestingly, circulating catecholamine levels were also found to be increased in two mouse models of viral myocarditis^{24,25}, suggesting the possibility that enhanced catecholamine release may also be an underlying cause of atrial involvement in lymphocytic myocarditis. Alternatively, viral infection could in theory underlie atrial myocarditis in patients with lymphocytic myocarditis. In a mouse model of coxsackievirus B3-

induced myocarditis, atrial myocarditis was also induced and coincided with high atrial virus titres²⁶. However, viral infection of the atria coinciding with viral infection of the ventricles in humans has not been reported yet. Unfortunately, in our patients with lymphocytic myocarditis, no culprit virus was determined. Finally, autoimmunity against cardiomyocytes, an important co-founder of cardiac damage and inflammation in myocarditis, may underlie atrial myocarditis also. Indeed, in a rat model of experimental autoimmune myocarditis, inflammatory lesions were observed in the atria as well²⁷. Thus, the exact mechanisms underlying the concomitant atrial myocarditis, in myocarditis of both aetiologies, remains to be determined.

The clinical relevance of the atrial myocarditis we have observed in our patients with myocarditis, remains to be established also. However, myocarditis is listed as a cardiac precursor for AF¹⁴ and AF is found in 14% of patients with suspected myocarditis²⁸. Furthermore, local and systemic inflammation have been implicated in the pathophysiology of AF, ranging from observed increased local and systemic production of pro-inflammatory cytokines^{10,29}, to increased inflammatory cell infiltration in the atria^{12,13,30} in patients with lone AF. Therefore, although the patients with myocarditis included in this study did not have symptomatic AF, the increase in atrial inflammatory cell infiltration may indicate a predisposition towards the development of AF. In light of this, our finding of increased inflammatory cell infiltration in the adipose tissue of the atria is of interest, because a role for atrial adipose tissue in AF has been postulated. Apart from associations between atrial epicardial fat volume and the occurrence and severity of AF^{31,32}, atrial adipose tissue may be an important source of pro-inflammatory bioactive molecules such as IL-6, tumour necrosis factor (TNF)- α and monocyte chemoattractant protein-1 (MCP-1), which may induce AF³³. Infiltrated inflammatory cells in theory, could add to this paracrine effect. Moreover, a recent study showed that the inflammatory activity, reflected by glucose metabolism, of epicardial adipose tissue was significantly increased in patients with AF³⁴. This is in agreement with our recent observations in the left atrial appendage tissue of AF patients, in which inflammatory cells were also found to infiltrate the adipose tissue (unpublished results).

As mentioned above, inflammation can induce the formation of atrial fibrosis, which increases the risk of AF development¹⁵. In our analysis, however, atrial inflammation did not coincide with an increase in atrial fibrosis. Because no increase was observed between patient groups, this analysis provides no further insight into whether the observed atrial inflammation precedes AF. However, the amount of collagen did differ between the left and right atria of the same patient group. It is known that the left and right atrium differ in morphology³⁵ and gene expression profile³⁶, however, to the best of our knowledge, this difference in intramyocardial collagen deposition has never been described before.

In conclusion, in this study we demonstrated that ventricular myocarditis coincides with atrial myocarditis. The observed atrial myocarditis may predispose patients to the development of

AF and subsequent complications, such as sudden cardiac death and heart failure. However, although the link between inflammation and AF has been firmly established, the causal relation between the two remains a topic of controversy. Prospective studies in living patients will be necessary to determine whether the observed atrial myocarditis indeed predisposes towards AF.

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Supplementary table 1. Correlations in lymphocytic myocarditis patients

	LA myo vs. LV myo	RA myo vs. RV myo	LA adi vs. LA myo	RA adi vs. RA myo
Neutrophilic granulocytes	r = 0.771 p = 0.103 cod = 59.4%	nc	r = 0.314 p = 0.564 cod = 9.86%	nc
Lymphocytes	nc	r = 0.886 p = 0.033 cod = 78.5%	nc	nc
Macrophages	r = 0.771 p = 0.103 cod = 59.4%	r = 0.943 p = 0.017 cod = 88.9%	r = 0.600 p = 0.350 cod = 36.0%	r = -0.600 p = 0.350 cod = 36.0%

adi, adipose tissue; cod, coefficient of determination; LA, Left atrium; LV, Left ventricle; myo, myocardium; nc, no correlation; p, p-value; RA, Right atrium; RV, Right ventricle; r, Spearman's rank correlation coefficient.

Supplementary table 2. Correlations in patients with catecholamine-induced myocarditis

	LA myo vs. LV myo	RA myo vs. RV myo	LA adi vs. LA myo	RA adi vs. RA myo
Neutrophilic granulocytes	r = 0.300 p = 0.683 cod = 9.0%	r = 0.900 p = 0.08 cod = 81.0%	r = 0.500 p = 0.450 cod = 25.0%	nc
Lymphocytes	nc	nc	nc	nc
Macrophages	r = 0.800 p = 0.133 cod = 64.0%	r = 0.600 p = 0.350 cod = 36.0%	r = 0.500 p = 0.450 cod = 25.0%	r = 0.500 p = 0.450 cod = 25.0%

adi, adipose tissue; cod, coefficient of determination; LA, Left atrium; LV, Left ventricle; myo, myocardium; nc, no correlation; p, p-value; RA, Right atrium; RV, Right ventricle; r, Spearman's rank correlation coefficient.